International Journal of Infectious Diseases 16 (2012) e414–e416

Contents lists available at SciVerse ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Perspective

Highly effective and inexpensive parasitological technique for diagnosis of intestinal parasites in developing countries: spontaneous sedimentation technique in tube

Raúl Tello [a,*], Angélica Terashima [a], Luis A. Marcos [a,b], Jorge Machicado [a], Marco Canales [a], Eduardo Gotuzzo [a]

[a] Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, San Martín de Porras, Lima 31, Peru
[b] Infectious Diseases Division, Washington University, St. Louis, Missouri, USA

ARTICLE INFO

Article history:
Received 3 October 2011
Received in revised form 20 November 2011
Accepted 31 December 2011

Corresponding Editor: Karamchand Ramotar, Ottawa, Canada

Keywords:
Intestinal parasites
Diagnosis
Sedimentation technique
Peru

SUMMARY

Millions of low-income people in the world are affected by intestinal parasites. Inexpensive, simple, and effective techniques for diagnosis are necessary. The spontaneous sedimentation technique in tube (SSTT), for application in poor healthcare settings and under field-work conditions, was described 25 years ago in Peru by Tello. The advantages of the SSTT are its ability to detect the majority of intestinal parasites, including eggs, larvae, cysts, and trophozoites, and its low cost.

1. Introduction

Several neglected tropical diseases are underestimated in developing countries for a variety of reasons, ranging from theoretical estimates, such as calculating the burden of disease using a mathematical formula (e.g., disability-adjusted life years – DALYs),[1] to more obvious practical reasons, such as a lack of screening diagnostic tests, limited access to healthcare facilities for the affected people, impractical report forms for the documentation of common diseases by healthcare providers in areas with limited accessibility (e.g., lack of computerized services), and a lack of more advanced tests such as molecular biology tools (e.g., PCR or antigen-based tests), which are costly for these poor communities and very unlikely to be implemented in the near future.

A major and worse scenario is when the reporting of data for a certain disease is insufficient, such that it does not fall under the definition of a so-called 'neglected' disease, since it may not exist in clinical practice because of a low prevalence or may not be a priority in a determined healthcare system (e.g., echinococcosis or fasciolisis in Latin America).[2] Furthermore, the number of people infected by intestinal parasites, not only soil-transmitted helminthiases, but also protozoa, tapeworms, Strongyloides, and liver flukes, may be underestimated based on the parasitological technique in current use. Nonetheless, major problems in the development of novel diagnostic techniques to detect a broader spectrum of intestinal parasites in developing countries are the lack of reproducibility of advanced techniques in rural areas (e.g., PCR, or antigen-based detection kits, or implementation of centrifugal procedures), where the health centers have minimal laboratory equipment, the lack of peripheral laboratories, and the need for point-of-care testing. Despite these limitations, another major issue arises when assessment of cure of infection after therapy is affected by the low sensitivity of the diagnostic test.

A major public health problem in Peru is not only the high prevalence of soil-transmitted helminthiases, but also other intestinal parasites.[3] For the above-mentioned reasons, there is an urgent need to improve detection rates of intestinal parasites in populations living in extreme poverty; however, such a diagnostic technique should be of low cost and high sensitivity for success within the context of a low-income population.

In 1988, a novel technique called the spontaneous sedimentation technique in tube (SSTT) was described by Tello[4] (Table 1) as an alternative to the FAUST technique (sulfate zinc flotation technique), which was then in use as the routine procedure at the

* Corresponding author.
E-mail address: angelica.terashima@upch.pe (R. Tello).

1201-9712/$36.00 – see front matter © 2012 International Society for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijid.2011.12.017

Table 1
Original protocol for the spontaneous sedimentation technique in tube (SSTT) described by Dr Raul Tello in 1988[4]

Procedure steps	Description
I. Homogenize stools[a]	• Approx. 10 g of feces is mixed with 10 ml of normal saline solution until homogenized (30–60 s)
II. Spontaneous sedimentation	• Pour the homogenate into a conical tube (13×2.5 cm; 50 ml capacity) using a filter (surgical gauze) at the top of the tube
	• Discard gauze and fill out tube with normal saline solution; close cap tightly
	• Shake for 30 s and leave the tube in the vertical position for 45 min
III. Sediment analysis	• Take the sediment from the bottom of the tube using a plastic pipette
	• Place 2–3 drops in two smears; add Lugol's solution to one of them, cover with cellophane (6×2 cm), and observe under a microscope ($100\times$ and $400\times$)

[a] It is very important to have the stools homogenized without large particles; it may take longer than 60 s as needed.

Institute of Tropical Medicine Alexander von Humboldt, Universidad Peruana Cayetano Heredia (UPCH), Lima, Peru. The SSTT was shown to have greater sensitivity and required less costly materials. A brief summary of the initial technique as described by Dr Tello is as follows: "a 50-ml shaped plastic tube and a surgical gauze were used to filter the homogenized stools of patients infected by intestinal parasites; after filling the tube with normal saline solution and allowing it to sediment for 1–2 h, eggs, larvae, and cysts/trophozoites of protozoa and common intestinal parasites were seen in all sediments". SSTT is called spontaneous because the homogenized filtered fecal material requires 30–45 min (the longer the better) to sediment spontaneously without mechanical intervention or centrifuges.

Over the next 10 years, the SSTT was found to be continuously superior and more effective in diagnosing intestinal parasites (both protozoa and helminthes) in comparison to the FAUST technique, and to be less expensive (Tello and Terashima, personal communication). In 1995, the SSTT was finally included into the set of routine parasitological techniques for the diagnosis of intestinal parasites at the Institute of Tropical Medicine Alexander von Humboldt. Its high efficacy for detecting intestinal parasites, including *Fasciola hepatica* and *Strongyloides stercoralis*, has been widely published in local journals.[5–15] More recently, an initiative among some parasitologists in Peru has recommended this technique be implemented and used in epidemiological studies in order to prevent an underestimation of the burden of intestinal parasites. Because of a favorable background for the use of the SSTT in field conditions and in poor healthcare settings, the objective of this study was to evaluate the performance of the SSTT when compared with other parasitological techniques.

2. Methods

The MedLine, LILACs, Google Scholar, LIPECs, and SciELO databases were searched for relevant information for the period 1988–2011. The inclusion criterion was any study in which the SSTT was compared to at least one additional parasitological technique. The data search was performed by two independent subjects (JM and LAM). Any disagreement was resolved by consensus. The Chi-square test or Fisher's exact test was performed for the comparison of proportions. A p-value of <0.05 was considered statistically significant. Statistical analysis was carried out using SPSS 18.0 for Windows (SPSS Inc., 2010).

3. Results

Out of 14 studies collected, only four met the inclusion criteria.[16–19] The SSTT was compared to direct smear exam, ether–formalin concentration method (EFCM), and sulfate zinc flotation technique. The SSTT reported higher prevalence rates than the direct smear test for all detected parasites, as follows: *Ascaris lumbricoides* (35% vs. 19%; $n = 1441$; $p = 0.0001$), *Trichuris trichiura* (22% vs. 6%; $n = 1441$; $p = 0.0001$), hookworm (7% vs. 3%; $n = 1441$; $p = 0.0001$), *Hymenolepis nana* (7% vs. 4%; $n = 1441$; $p = 0.0001$), *Enterobius vermicularis* (1.5% vs. 0.1%; $n = 1441$; $p = 0.0001$), *Strongyloides stercoralis* (7% vs. 3%; $n = 1441$; $p = 0.0001$), *Blastocystis hominis* (6% vs. 3%; $n = 1441$; $p = 0.0001$), *Giardia lamblia* (7% vs. 4%; $n = 1441$; $p = 0.0008$), *Cyclospora cayetanensis* (4% vs. 2%; $n = 108$; $p = 0.6$), and *Entamoeba histolytica/Entamoeba dispar* (1% vs. 0.5%; $n = 1333$; $p = 0.09$). When compared to EFCM in 194 samples, the SSTT also reported higher prevalence rates for all detected parasites: *A. lumbricoides* (8% vs. 5%; $p = 0.3$), *T. trichiura* (10% vs. 5%; $p = 0.07$), hookworm (0.5% vs. 0.5%; $p = 1$), *H. nana* (18% vs. 11%, $p = 0.08$), *E. vermicularis* (2% vs. 1%; $p = 0.6$), *Fasciola hepatica* (13% vs. 8%; $p = 0.1$), and *B. hominis*. (55% vs. 41%; $p = 0.008$). Similarly, when compared to the sulfate zinc flotation technique, the SSTT had higher detection rates for all detected parasites: *A. lumbricoides* (6% vs. 4%; $p = 0.5$), *T. trichiura* (0.9% vs. 0%), hookworm (3% vs. 2%; $p = 1$), *H. nana* (5% vs. 3%; $p = 0.7$), *E. vermicularis* (4% vs. 3%; $p = 1$), *F. hepatica* (0.9% vs. 0%), *B. hominis* (34% vs. 3%; $p = 0.001$), *G. lamblia* (10% vs. 9%; $p = 1$), and *C. cayetanensis* (4% vs. 2%; $p = 0.3$). No studies have compared the

Table 2
Summary of the comparison between the spontaneous sedimentation technique in tube (SSTT) and the direct smear exam, ether–formalin concentration method, and sulfate zinc flotation technique

Parasites	SSTT vs. direct smear test ($n = 1441$)	SSTT vs. EFCM ($n = 194$)	SSTT vs. SZFT ($n = 108$)
Helminths			
Ascaris lumbricoides	35% vs. 19%; $p < 0.001$	8% vs. 5%; $p > 0.05$	6% vs. 4%; $p > 0.05$
Trichuris trichiura	22% vs. 6%; $p < 0.001$	10% vs. 5%; $p > 0.05$	0.9% vs. 0%; $p > 0.05$
Hookworm	7% vs. 3%; $p < 0.001$	0.5% vs. 0.5%; $p > 0.05$	3% vs. 2%; $p > 0.05$
Hymenolepis nana	7% vs. 4%; $p < 0.001$	18% vs. 11%; $p > 0.05$	5% vs. 3%; $p > 0.05$
Enterobius vermicularis	1.5% vs. 0.1%; $p < 0.001$	2% vs. 1%; $p > 0.05$	4% vs. 3%; $p > 0.05$
Strongyloides stercoralis	7% vs. 3%; $p < 0.001$	NA	NA
Fasciola hepatica	NA	13% vs. 8%; $p > 0.05$	0.9% vs. 0%; $p > 0.05$
Protozoa			
Blastocystis hominis	6% vs. 3%; $p < 0.001$	55% vs. 41%; $p < 0.05$	34% vs. 3%; $p < 0.001$
Giardia lamblia	7% vs. 4%; $p < 0.001$	NA	10% vs. 9%; $p > 0.05$
Cyclospora cayetanensis	4% vs. 2% ($n = 108$; NS)	NA	4% vs. 2%; $p > 0.05$
Entamoeba histolytica/Entamoeba dispar	1% vs. 0.5% ($n = 1333$; NS)	NA	NA

EFCM, ether-formalin concentration method; SZFT, sulfate zinc flotation technique; NS, not significant or $p > 0.05$; NA, data not available from the original studies.

SSTT with the Kato–Katz or FLOTAC techniques. A summary of the comparisons between the SSTT and the other techniques is presented in Table 2.

4. Conclusions

Based on these findings, the potential of the SSTT as a diagnostic tool for intestinal parasites in endemic areas is promising, but further studies are required (e.g., comparison with Kato–Katz technique and other techniques). The major advantages of the SSTT technique are its relatively low material cost (approx. US$0.03) and its simplicity of use in poor healthcare settings; both of these aspects were built into Tello's original idea for developing an alternative diagnostic strategy 25 years ago.

Conflict of interest: The authors declare that they have no competing interests.

References

1. King CH, Bertino AM. Asymmetries of poverty: why global burden of disease valuations underestimate the burden of neglected tropical diseases. *PLoS Negl Trop Dis* 2008;**2**:e209.
2. Dujardin JC, Herrera S, do Rosario V, Arevalo J, Boelaert M, Carrasco HJ, et al. Research priorities for neglected infectious diseases in Latin America and the Caribbean region. *PLoS Negl Trop Dis* 2010;**26**:1–5.
3. Cabrera R. Helmintos intestinales en el Peru: análisis de la prevalencia (1981–2001). Peru: Oficina General de Epidemiologia, Ministerio de Salud; 2003.
4. Tello R. Empleo de una nueva técnica parasitológica rápida de sedimentación espontánea en el diagnóstico de protozoarios y helmintos. Resumen. V Jornadas Científicas. II Jornadas Científicas Estudiantiles. Lima, Peru; 1988, p. I–164.
5. Tello R, Canales M. Técnicas de diagnóstico de enfermedades causadas por enteroparásitos. *Diagnóstico* 2000;**39**:197–8.
6. Maco V, Marcos L, Terashima A, Tello R, Samalvides F, Gotuzzo E. Técnica de sedimentación espontánea en tubo (TSET): alto rendimiento en el diagnóstico de parásitos intestinales en comparación con otras técnicas coproparasitológicas. *Rev Med Hered* 2001;**12**:111.
7. Marcos L, Maco V, Terashima A, Samalvides F, Miranda E, Gotuzzo E. Parasitosis intestinal en poblaciones urbana y rural en Sandia, Departamento de Puno, Perú. *Parasitol Latinoam* 2003;**58**:35–40.
8. Marcos LA, Maco V, Terashima A, Samalvides F, Gotuzzo E. Características clínicas de la infección crónica por *Fasciola hepatica* en niños. *Rev Gastroenterol Peru* 2002;**22**:228–33.
9. Marcos LA, Maco V, Terashima A, Samalvides F, Miranda E, Tantalean M, et al. Hiperendemicidad de fasciolosis humana en el Valle del Mantaro: factores de riesgo de la infección por *Fasciola hepatica*. *Rev Gastroenterol Peru* 2004;**24**:158–64.
10. Marcos LA, Maco V, Castillo M, Terashima A, Serpa R, Gotuzzo E. Reporte de casos de fasciolosis en el Instituto Especializado de Salud del Niño (1988–2003). *Rev Gastroenterol Peru* 2005;**25**:198–205.
11. Marcos LA, Maco V, Samalvides F, Terashima A, Espinoza JR, Gotuzzo E. Risk factors for *Fasciola hepatica* infection in children: a case–control study. *Trans R Soc Trop Med Hyg* 2006;**100**:158–66.
12. Marcos LA, Terashima A, Leguia G, Canales M, Espinoza JR, Gotuzzo E. *Fasciola hepatica* infection in Peru: an emergent disease. *Rev Gastroenterol Peru* 2007;**27**:389–96.
13. Marcos LA, Romani L, Florencio L, Terashima A, Canales M, Nestares J, et al. Zonas hiperendémicas y mesoendémicas de la infección por *Fasciola hepatica* aledañas a la ciudad de Lima: una enfermedad emergente? *Rev Gastroenterol Peru* 2007;**27**:21–6.
14. Marcos LA, Terashima A. Update on human fascioliasis in Peru: diagnosis, treatment and clinical classification proposal. *Neotropical Helminthology* 2007;**1**:85–103.
15. Marcos LA, Tagle M, Terashima A, Bussalleu A, Ramirez C, Carrasco C, et al. Natural history, clinicoradiologic correlates and response to triclabendazole in acute massive fascioliasis. *Am J Trop Med Hyg* 2008;**78**:222–7.
16. Pajuelo-Camacho G, Luján-Roca D, Paredes-Pérez B, Tello-Casanova R. Aplicación de la técnica de sedimentación espontánea en tubo en el diagnóstico de parásitos intestinales. *Rev Biomed* 2006;**17**:96–101.
17. Terashima A, Marcos LA, Maco V, Canales M, Samalvides F, Tello R. Técnica de sedimentación en tubo de alta sensibilidad para el diagnostico de enteroparasitos. *Rev Gastroenterol Peru* 2009;**4**:305–10.
18. Lau-Chong C, Samalvides F, Terashima A. Evaluación de técnicas parasitológicas en el diagnóstico de estrongiloidiasis por *Strongyloides stercoralis*. *Rev Med Hered* 2005;**16**:11–8.
19. Vasquez F. *Giardia lamblia*: estudio comparativo de tres métodos de diagnostico, examen directo de heces, enterotest modificado y técnica de sedimentación espontanea en tubo. Peru: Instituto de Investigación, Facultad de Medicina Humana USMP; 1997. Available at: http://www.usmp.edu.pe/medicina/horizonte/1997/Art2_Vol1_N2.pdf (accessed March 6, 2011).